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Applicant #1, Name: Rong Jian Yang

Applicant #2, Name: Shing Paau

Title: IgY Against Dental Caries and Dental Caries-Preventive Combination

☒ Specification, Claims, and Abstract: Nr. Of Sheets 10

☒ Declaration: Date Signed: 07/08/2000

☒ Drawing(s): Nr. Of Sheets Enc.: (In Triplicate): Formal: \_\_\_\_\_ Informal: \_\_\_\_\_

☒ Small Entity Declaration Of Inventor(s) ☐ SED of Non-Inventor / Assignee / Licensee

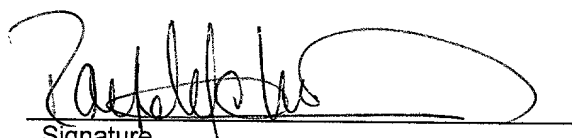
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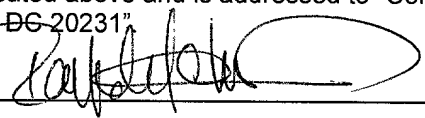
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Signature  
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Reg. Nr. : 37,484  
1050 Oakdale Lane  
Arcadia, CA 91006

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Applicant or Patentee: \_\_\_\_\_ Attorney's  
Serial or Patent No.: \_\_\_\_\_ Docket No.: USP1122A-JMG  
Filed or Issued: \_\_\_\_\_  
For: \_\_\_\_\_

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
STATUS (37 CFR 1.9 (f) and 1.27 (b)) - INDEPENDENT INVENTOR**

As a below named inventor, I hereby declare that I qualify as an independent inventor as defined in 37 CFR 1.9 (c) for purposes of paying reduced fees under section 41 (a) and (b) of Title 35, United States Code, to the Patent and Trademark Office with regard to the invention entitled:  
lgY Against Dental Caries and Dental Caries-Preventive Combination described in:

☒ the specification filed herewith  
☐ application serial no. \_\_\_\_\_, filed \_\_\_\_\_  
☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_

I have not assigned, granted, conveyed or licensed and am under no obligation under contract or law to assign, grant, convey or license, any rights in the invention to any person who could not be classified as an independent inventor under 37 CFR 1.9 (c) if that person had made the invention, or to any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a non profit organization under 37 CFR 1.9 (e).

Each person, concern or organization to which I have assigned, granted, conveyed, or licensed or am under an obligation under contract or law to assign, grant, convey, or license any rights in the invention is listed below:

☐ no such person, concern, or organization  
☒ person, concern or organizations listed below\*

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

FULL NAME Jason Medical Holding, Inc. (USA)  
ADDRESS 475 El Camino Real, Suite 218, Millbrae, CA 94030, USA  
☐ INDIVIDUAL ☒ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

FULL NAME \_\_\_\_\_  
ADDRESS \_\_\_\_\_  
☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statements is directed.

Rong Jian YANG Shing PAAU \_\_\_\_\_  
NAME OF INVENTOR NAME OF INVENTOR NAME OF INVENTOR

Rong Jian Yang [Signature] \_\_\_\_\_  
Signature of Inventor Signature of Inventor Signature of Inventor

Date July 8, 2000 Date July 8, 2000 Date \_\_\_\_\_

Applicant or Patentee: \_\_\_\_\_ Attorney's  
Serial or Patent No.: \_\_\_\_\_ Docket No.: USP1122A-JMG  
Filed or Issued: \_\_\_\_\_  
For: \_\_\_\_\_

**VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY  
STATUS (37 CFR 1.9 (f) and 1.27 (e)) - SMALL BUSINESS CONCERN**

I hereby declare that I am

- ☒ the owner of the small business concern identified below:  
☐ an official of the small business concern empowered to act on behalf of the concern identified below:

Name of Concern: Jason Medical Holdings, Inc. (USA)

Address of Concern: 475 El Camino Real, Suite 218, Millbrae, Ca 94030, USA

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9 (d), for purposes of paying reduced fees under section 41(a) and (b) of title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees is the average over the previous fiscal year of the concern of the persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power of control the other, or a third party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled:

IgY Against Dental Caries Bacteria and Dental Caries-Preventive Combination  
described in:

- ☒ the specification filed herewith  
☐ application serial no. \_\_\_\_\_, filed \_\_\_\_\_  
☐ patent no. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the above identified small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9(d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as a small entities. (37 CFR 1.27)

Full Name:

Address: ☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NON PROFIT ORGANIZATION

Full Name:

Address: ☐ INDIVIDUAL ☐ SMALL BUSINESS CONCERN ☐ NON PROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statements is directed.

Name of Person Signing: Shing PAU

Title of Person Other than Owner: Executive Director

Address of Person Signing: 475 El Camino Real, Suite 218, Millbrae, Ca 94030, USA

Signature

Date

8-7-2000

## Title

### **IgY Against Dental Caries and Dental Caries – Preventive Combination**

## Background of the Present Invention

### **Field of Invention**

5           The invention is put under preparation of immunoglobulin from hen yolk, especially, IgY against dental caries bacteria and the combination preventing dental caries wherein the IgY and antiseptic are as effective components.

### **Description of Related Arts**

10           It is well know that streptococcus mutans are major dental caries bacteria. There are two measures of passive immunization to streptococcus mutans at the moment. First, cows or hens are immunized with single or mixed streptococcus mutans as antigen, the antibodies are extracted from milk or yolk, then passive immunization is taken place. Second, cows or hens are immunized with glucosyl transferase of streptococcus mutans as antigen, the following steps are as same as those of the first. There are, however, 15 problems for available preparative technique of antibody: 1. high cost of antibody preparation, especially, difficulty of antigen extract while glucosyl transferasse is used as antigen. 2. low titer of antibody, the highest titer is only 1:320 by existing preparative technique. 3. The yolk could not be comprehensively used.

### **Summary of the Present Invention**

20           The purpose of present invention raises a preparative method of IgY against dental caries, which reduces cost of production, elevates titer of antibody, and yolk can be utilized in multi-propose, simultaneously, provides dental caries preventing combinations where IgY of the invention and antiseptic are as effective components.

The preparative method of IgY against dental caries in the invention is as the following:

Streptococcus mutans type c and type d are separately grown BHI or TTY culture medium for 2-3 days centrifuged to collect the bacteria. The bacteria are rinsed 4-6 times with 0.05-0.2 M of phosphate buffered saline, pH 6-7, heated at 50-60°C for 25-35 minutes. To prepare antigens, mix streptococcus mutans type c and type d by ratio 1-2:1, add Freund' s adjuvant equal to total volume of both bacteria, then treated with high speed homogenized.

The best ratio of type c and type d mixture is 2:1.

The hens are immunized by three hypodermic or wing vein injections, 1.0ml (1x10<sup>9</sup>/ml) of streptococcus mutans each time, at 2weeks intervals. Yolks are taken out by sieve, stirred even, diluted by adding 4-6 fold of distilled water. Adjust pH to 4.5-6.5, stand at 3-5°C for 20-30 hours, centrifuge at high speed for 20-30 minutes. The supernatant is ultra filtrated, followed by sterilization and lyophilization. This is crude IgY extract against dental caries.

The crude extract is applied on DEAE-Sephadex A50 column, eluted with phosphate buffer containing 0.03-0.1M of NaCl by gradient elution followed by pouring protein peaks, estimating antibody activity with ELISA, and adjusting active eluates to 20mg protein /ml.

Obtained eluates are applied on Sephadex G200 column, eluted with phosphate buffer containing 0.05-0.2M of NaCl by gradient elution followed by pouring protein peaks, estimating antibody activity with ELISA, sterilizing by 0.22M membrane filtration, and lyophilizing. T is purified IgY against dental caries bacteria.

The best concentrations of NaCl in phosphate butter eluants for DEAE-Saphadex A50 and Sephadex G200 are 0.07M and 0.1M, respectively.

Based on the following results, present invention chooses streptococcus mutans type c and type d as antigen bacteria.

Streptococcus mutans have serotype a, b, c, d, e, f, g, and h, etc. in accordance with serum typing. In oral cavity, however, type c type d account for about 60-90%and10%, respectively, the others are very low, therefore, type c and type d are major serotype bacteria causing dental caries. Choosing type c and type d as antigen bacteria advantage in either avoiding reducing effectively of major serotype bacteria when mixture of multi- serotype bacteria is used as antigens, or avoiding narrow range of immunological cross reaction when single serotype bacteria is used as antigens. Moreover, the investigations indicate that protein antigen A and B can be extracted from cell wall of streptococcus mutans of oral cavity, type c has both protein A and B, type b has only protein A type a, d, e, and g have only protein B, thus, type c and d as antigens insure that the antibodies have wide range of cross reaction.

The invention adopts water dilution to extract IgY, which comprehensively utilizes of the yolks with low cost, simple technology and no environmental pollution.

IgY of present invention has reached PAGE purity with 180 kD of molecular weight by SDS-PAGE.

The experiments showed that the IgY keeps its activity during 90 minutes at 65°C; its activity has no significant change after 8 hours at 37°C, but rapidly decreased, then inactivated at pH 2.0 or pH 12.0; the IgY is resistant to osmotic pressure, for instance, tolerant to 40% sucrose.

The IgY of the invention can effectively inhibit agglutination of streptococcus mutans by indirect hem agglutination test with the titer I:51, obviously inhibit adhesion of the bacteria until it is diluted to 1:8; animal experiments takes that IgY can effectively prevent occurrence of dental caries by feeding IgY rats infected with streptococcus mutans, the bacteria number in bacteria plaques reduce 70-80%, the results of contrast experiment of dental caries formation in rats is expressed in the following table:

	Control	Experiment	P Value
I	47	19	< 0.01
II	25	0.57	< 0.01
III	8	0	< 0.01

I: only damage of enamel; II: damaged ¼ of denting; III: damaged through dentine. Dental caries score by Keyes method.

The difference between two groups is very significant ( $p < 0.01$ ).

The dental caries preventing combination means that the combination's effective components are IgY of present invention and antiseptic, the later is, at least, one of both potassium sorbate and sodium benzoate, the combination may be either a product for oral cavity use, for example, toothpaste, buccal liquid, mouthwash, or food, for instance, chewing gum, chocolate, ice cream, milk (powder), bean milk (powder), etc. Additive amount of the IgY usually is 0.05-0.2%, potassium sorbate or sodium benzoate is 0.005-0.02%.

The combination can be packaged in pocket atomizer when as liquid product used in oral cavity, and in sucking bottle as food for serving.

Coordinated with decontaminaters and ozostomia preventer, diverse products can be manufactured of the IgY for prevention and treatment. Also, it can be added in oral cavity frushres to enhance their function preventing dental caries.

The IgY of the invention is low cost of preparation and production, high titer of antibody, resistant to osmotic pressure, strong immunological activity and wide range of cross-reaction to streptococcus mutans. The combination of present invention features in small amount of IgY, safe use, effective prevention and treatment, etc. It can effectively prevent occurrence of dental caries.

### Detailed Description of the Preferred Embodiment

The invention will be described further by the following samples:

Sample 1 Streptococcus mutans type c and type d are separately cultivated in BHI medium at 37°C for 48 hours, followed by collecting bacteria with centrifugation, at 4000 rpm for 10 minutes, winsing 5 times with 0.2M of phosphate buffered saline, pH 6.0, and heating at 50°C for 25 minutes. Each of type c and type d is adjusted to  $2 \times 10^8$  /ml of the suspensions. Mix equal volume of type c and type d, then, Freund's adjuvant equal to total volume of type c and type d is added in it then, homogenized at high speed. This is a streptococcus mutans antigen.





phosphate buffer containing 0.06M of NaCl, 20ml/h. 5.0ml each fraction; pour each peas, estimate antibody activity with ELISA. Keep the active eluates, sterilize by 0.22 µm membrane and pyophilize.

#### Preparation of product of the combination preventing dental caries.

5            Sample 3    Preparation of IgY buccal liquid    Take 2.0g of the IgY, 0.15g of potassium sorbate, 0.8g of sugar, and 0.15g of menthol and 0.4ml of apple essence. Add menthol into 100ml of distilled water and dissolved at 60°C, other solid components are dissolved in 450ml of distilled water, combine both solutions, then add distilled water to 1 000ml.

10           Sample 4    Preparation of chewing gum    Take 2.0g of the IgY, 0.15g of potassium sorbate, 0.8g of sugar, 0.15g of menthol, 0.4ml of apple essence, 10.08g base and 5.08g of CM-cellulose, then add substrate material to 1 000g.

15           Sample 5    Preparation of IgY toothpaste    Take 0.1g of the IgY, 0.015g of potassium sorbate, 0.015g of sodium benzoate, 10.0g of glycerol, 8.0g of sorbitol, 2.0g of CM-cellulose, 1.3g of sodium trehalate, 1.8g of sodium lauryl sulfate, 0.015g of menthol, 0.015g of sugar, 0.05ml of strawberry essence, 47.8g of calcium phosphate. 2H<sub>2</sub>O. Swell CM-cellulose to dissolve followed by orderly adding other components, stir thoroughly, add distilled water to 1 000ml, and then stir until it becomes paste.

20           Sample 6    IgY tooth- protecting paste    Take 0.1g of the IgY, 0.01g of sodium benzoate, 8.0g of beeswax, 10.0g of Stearic acid, 2.0g of monostearyl glyceride, 10.0g of glycerol, 1.0g of CM-cellulose, 0.01g of menthol, 0.05g of sugar, 68.80ml of distilled water and 0.02g of strawberry essence. Mix beeswax, Stearic acid, monostearyl glyceride and glycerol, and heat to 70°C, named solution A. Swell CM-cellulose in 50ml distilled water to dissolve, orderly and IgY, menthol, sugar, potassium sorbate, and streaberry  
25           essence, stir thoroughly, then add cooled solution a, add distilled water to 100ml, stir until it becomes paste.

30           Sample 7    Preparation of IgY nutrient milk    Add IgY of the invention and potassium sorbate, whose final concentrations are 0.1% and 0.015%, respectively, into pasteurized frush milk, homogenize with sterile homogenized, pour into sterile sucking bottles, store at 4°C.

Sample 8 Preparation of IgY nutrient milk powder Take IgY of present invention and potassium sorbate, whose final concentrations are 0.1% and 0.005%, respectively, in pasteurized fresh milk powder, mix with sterile mixed, package sterily in bags.

- 5 Sample 9 Preparation of IgY nutrient bean milk Add IgY of the invention and sodium benzoate, whose final concentrations are 0.1% and 0.05%, respectively in pasteurized compounded bean milk, homogenize with sterile homogenized, pour into sterile sucking bottles, store at 4°C.

- 10 Sample 10 Preparation of IgY nutrient bean milk powder Add IgY of the invention and sodium benzoate, whose final concentrations are 0.1% and 0.005%, respectively, in pasteurized bean milk powder, mix with sterile mixer, sterily package in bags.

What is Claimed is:

1. A preparation method of immunoglobulin Y (IgY) against dental caries bacteria, including the steps of preparing streptococcus mutans antigens which are streptococcus streptococcus mutans type c and type d as antigen bacteria, immunizing  
5 hens, and extracting IgY by water dilution, DEAE-Sephadex A50 and Sephadex G200 chromatography.
2. The preparation method as recited in claim 1 wherein said streptococcus mutans type c and d as antigen bacteria are mixed by ratio 2:1.
3. The preparation method as recited in claim 1 wherein in the extracting step  
10 further includes the steps of extracting IgY by water dilution, adding 4-6 fold distilled water into even yolk by stirring, adjusting pH to 4.5-6.5, stand at 3-5°C, and centrifuging at high speed for 20 minutes.
4. The preparation method as recited in claim 1 wherein an eluant which is a phosphate buffer containing 0.03-0.1M of NaCl is used for DEAE-Sephadex A50  
15 chromatography.
5. The preparation method as recited in claim 1 wherein an eluant which is a phosphate buffer containing 0.07M of NaCl is used for DEAE-Sephadex A50 chromatography.
6. The preparation method as recited in claim 1 wherein an eluant which is a  
20 phosphate buffer containing 0.05-0.2M of NaCl is used for Sephadex G200 chromatography.
7. The preparation method as recited in claim 1 wherein an eluant which is a phosphate buffer containing 0.1M of NaCl is used for Sephadex G200 chromatography.
8. A combination against dental caries bacteria, comprising an IgY to dental  
25 caries bacteria and, at last, one of both potassium sorbate and sodium benzoate.

9. The combination as recited in claim 8 wherein an additive amount of said IgY is at least 0.05% and an additive amount potassium sorbate and sodium benzoate is 0.005-0.02%.

10. The combination as recited in claim 9 wherein an additive amount of said  
5 IgY is 0.05-0.2%.

11. The combination as recited in claim 8 wherein said combination is packaged in pocket atomizer for spraying usage.

12. The combination as recited in claim 8 wherein said combination is made in liquid form and packaged in a sucking bottle.

## **IgY Against Dental Caries and Dental Caries – Preventive Combination**

### **Abstract of the Disclosure**

For the purpose of preparing the immunoglobulin of yolk (IgY) against dental caries bacteria and the combination, Streptococcus mutans type c and type d are used as antigen bacteria, the antibody is purified by water dilution, DEAE-Sephadex A50 and Sephadex G200 chromatography. It is low cost of production, high titer, strong resistance to osmotic pressure, and high immune activity and wide range of cross-reaction to streptococcus mutans. Present invention provides the combination preventing dental caries wherein the effective components are IgY and antiseptic features in safe use and effective prevention; The combination is effectively preventing dental caries.

## DECLARATION FOR PATENT APPLICATION

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

IgY Against Dental Caries and Dental Caries - Preventive Combination

the specification of which is attached hereto unless the following box is checked:

☐ was filed on \_\_\_\_\_ as United States Application Number or PCT International Application Number \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 USC 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

Prior Foreign Application(s)

Priority Claimed

☐ Yes ☐ No

\_\_\_\_\_  
(Number) (Country) (Day/Month/Year Filed)

☐ Yes ☐ No

\_\_\_\_\_  
(Number) (Country) (Day/Month/Year Filed)

☐ Yes ☐ No

\_\_\_\_\_  
(Number) (Country) (Day/Month/Year Filed)

I hereby claimed the benefit under 35 USC 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of 35 USC 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

\_\_\_\_\_  
(Application Number) (Filing Date) (Status-patented, pending, abandoned)

\_\_\_\_\_  
(Application Number) (Filing Date) (Status-patented, pending, abandoned)

I hereby appoint the following attorney(s) and/or agent(s), with full powers of substitution and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

**Raymond Yat Chiu Chao, Reg. No. 37,484**

Address all correspondence to: 1050 Oak Dale Lane, Arcadia, CA 91006, USA

Telephone Calls to: (626) 571-9812

I hereby declare that all Statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor (given name, family name) Rong Jian YANG

Inventor's signature Rong Jian Yang Date July 8, 2000  
Residence Same as below Citizenship Hong Kong, PRC  
Post Office Address Rm A6 Fl., Success Commercial Blvd., Hemessy Rd., Wan Chai, Hong Kong

Full name of second joint inventor, if any (given name, family name) Shing PAAU

Second Inventor's signature [Signature] Date July 8, 2000  
Residence Same as below Citizenship Hong Kong, PRC  
Post Office Address Unit 1205, 12/FI., Sino Plaza, 255 Gloucester Rd., Causeway Bay, Hong Kong

☐ Additional inventors are being named on separately numbered sheets attached hereto.